

SEQ List.ST25  
SEQUENCE LISTING

<110> Shanghai Hua-Yi Bio-Tech Lab  
 Sun, Yukun  
 Wu, Dengxi  
 Wu, Aizhen  
 Zhu, Zhiyong  
 Yu, Gang  
 Zhou, Jiaxiang  
 Zhao, Shaoling

<120> A Method of Producing Insulinotropic GLP-1 (7-36) Polypeptide  
 and/or GLP-1 Analogs

<130> 291-0002US

<150> CN01126278.8  
 <151> 2001-07-19

<150> PCT/CN02/00502  
 <151> 2002-07-17

<160> 31

<170> PatentIn version 3.2

<210> 1  
 <211> 30  
 <212> PRT  
 <213> homo sapiens

<400> 1

His	Ala	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Val	Ser	Ser	Tyr	Leu	Glu	Gly
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Gln	Ala	Ala	Lys	Glu	Phe	Ile	Ala	Trp	Leu	Val	Lys	Gly	Arg
			20					25					30

<210> 2  
 <211> 30  
 <212> PRT  
 <213> homo sapiens

<220>  
 <221> MOD\_RES  
 <222> (30)..(30)  
 <223> AMIDATION, Position 30 is Arg-NH2

<400> 2

His	Ala	Glu	Gly	Thr	Phe	Thr	Ser	Asp	Val	Ser	Ser	Tyr	Leu	Glu	Gly
1				5					10					15	

Gln	Ala	Ala	Lys	Glu	Phe	Ile	Ala	Trp	Leu	Val	Lys	Gly	Arg
			20					25					30

<210> 3

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<211> 31  
 <212> PRT  
 <213> homo sapiens

<400> 3

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
 1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly  
 20 25 30

<210> 4  
 <211> 30  
 <212> PRT  
 <213> Artificial

<220>  
 <223> This sequence contains one or more substituted amino acids  
 relative to the wild-type GLP-1 (7-36) sequence.

<400> 4

His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
 1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg  
 20 25 30

<210> 5  
 <211> 30  
 <212> PRT  
 <213> Artificial

<220>  
 <223> This sequence contains one or more substituted amino acids  
 relative to the wild-type sequence.

<400> 5

His Val Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
 1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg  
 20 25 30

<210> 6  
 <211> 30  
 <212> PRT  
 <213> artificial

<220>  
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 relative to the wild-type sequence.

<400> 6

SEQ List.ST25

His Ala Glu Gly Asp Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg  
20 25 30

<210> 7  
<211> 30  
<212> PRT  
<213> artificial

<220>  
<223> This sequence contains one or more substituted amino acids  
relative to the wild-type sequence.

<400> 7

His Ala Glu Gly Thr Phe Thr Ser Asp Ala Ser Ser Tyr Leu Glu Gly  
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg  
20 25 30

<210> 8  
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<212> PRT  
<213> artificial

<220>  
<223> This sequence contains one or more substituted amino acids  
relative to the wild-type sequence.

<400> 8

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Glu  
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Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg  
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<210> 9  
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<213> artificial

<220>  
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relative to the wild-type sequence.

<400> 9

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
1 5 10 15

His Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg  
20 25 30

SEQ List.ST25

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 <212> PRT  
 <213> artificial

<220>  
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 relative to the wild-type sequence.

<400> 10

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 1 5 10 15

Gln Glu Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg  
 20 25 30

<210> 11  
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 <212> PRT  
 <213> artificial

<220>  
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<400> 11

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
 1 5 10 15

Gln Ala Ala Trp Glu Phe Ile Ala Trp Leu Val Lys Gly Arg  
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<210> 12  
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 <212> PRT  
 <213> artificial

<220>  
 <223> This sequence contains one or more substituted amino acids  
 relative to the wild-type sequence.

<400> 12

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
 1 5 10 15

Gln Ala Ala Lys Ala Phe Ile Ala Trp Leu Val Lys Gly Arg  
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<210> 13  
 <211> 30  
 <212> PRT  
 <213> artificial

SEQ List.ST25

<220>  
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<400> 13

His Ala Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
 1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Glu Trp Leu Val Lys Gly Arg  
 20 25 30

<210> 14  
 <211> 30  
 <212> PRT  
 <213> artificial

<220>  
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<400> 14

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 1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Asp Lys Gly Arg  
 20 25 30

<210> 15  
 <211> 30  
 <212> PRT  
 <213> Artificial

<220>  
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<400> 15

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 1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Glu Gly Arg  
 20 25 30

<210> 16  
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 <212> PRT  
 <213> artificial

<220>  
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<400> 16

SEQ List.ST25

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1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Thr Arg  
20 25 30

<210> 17  
<211> 30  
<212> PRT  
<213> artificial

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<223> This sequence contains one or more substituted amino acids relative to the wild-type sequence.

<400> 17

His Gly Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
1 5 10 15

Gln Gly Ala Lys Glu Phe Ile Ala Trp Leu Val Lys Gly Arg  
20 25 30

<210> 18  
<211> 30  
<212> PRT  
<213> artificial

<220>  
<223> This sequence contains one or more substituted amino acids relative to the wild-type sequence.

<400> 18

His Leu Glu Gly Thr Phe Thr Ser Asp Val Ser Ser Tyr Leu Glu Gly  
1 5 10 15

Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Ala Lys Gly Arg  
20 25 30

<210> 19  
<211> 60  
<212> DNA  
<213> Artificial

<220>  
<223> GLP-1 synthetic sequence

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<210> 20  
<211> 62  
<212> DNA  
<213> artificial

# SEQ List.ST25

<220>  
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 <400> 20  
 accttcaga tagctgctca catcgctggt gaaggtacct tccgcgtgac gcatagatct 60  
 gg 62

<210> 21  
 <211> 58  
 <212> DNA  
 <213> Artificial

<220>  
 <223> GLP-1 synthetic sequence  
 <400> 21  
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<210> 22  
 <211> 56  
 <212> DNA  
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<220>  
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 <400> 22  
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<210> 23  
 <211> 60  
 <212> DNA  
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<220>  
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 <400> 23  
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<210> 24  
 <211> 62  
 <212> DNA  
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<220>  
 <223> GLP-1 synthetic sequence  
 <400> 24  
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 gg 62

<210> 25  
 <211> 58  
 <212> DNA

SEQ List.ST25

<213> Artificial

<220>

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<400> 25

gaaggtcagg cggcgaaaga atttatcgcg tggctggtga aaggtcgtct cgagtaga 58

<210> 26

<211> 56

<212> DNA

<213> Artificial

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<223> GLP-1 synthetic sequence

<400> 26

agcttctact cgagacgacc tttcaccagc cacgcgataa attctttcgc cgcttg 56

<210> 27

<211> 60

<212> DNA

<213> Artificial

<220>

<223> GLP-1 synthetic sequence

<400> 27

aattccagat ctatgatgca cgcggaaggt accttcacca gcgatgtgag cagctatctg 60

<210> 28

<211> 62

<212> DNA

<213> Artificial

<220>

<223> GLP-1 synthetic sequence

<400> 28

accttcaga tagctgctca catcgctggt gaaggtacct tccgcgtgca tcatagatct 60

gg 62

<210> 29

<211> 811

<212> DNA

<213> Artificial

<220>

<223> This sequence contains eight copies of GLP-1(7-36) polypeptide

<220>

<221> exon

<222> (13)..(807)

<400> 29

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## SEQ List.ST25

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agc Ser	tat Tyr 15	ctg Leu	gaa Glu
ggt Gly	cag Gln 20	gcg Ala	gaa Glu
ttt Phe 25	atc Ile 25	gcg Ala	tgg Trp
ctg Leu	gtg Val		99
aaa Lys 30	ggt Gly	cgt Arg	gga Gly
tct Ser 35	cgt Arg 35	cac His	gcg Ala
gaa Glu	ggt Gly	acc Thr 40	ttc Phe
acc Thr 45	agc Ser	gat Asp	gtg Val 45
147			
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gaa Glu 50	ggt Gly	cag Gln	gcg Ala
gaa Glu 55	ttt Phe 55	atc Ile	gcg Ala
tgg Trp 60	ctg Leu		195
gtg Val	aaa Lys	ggt Gly	cgt Arg 65
gga Gly	tct Ser	cgt Arg	cac His
gaa Glu 70	ggt Gly	acc Thr	ttc Phe
acc Thr 75	agc Ser	gat Asp	
243			
gtg Val	agc Ser 80	agc Ser	tat Tyr
ctg Leu	gaa Glu	ggt Gly 85	cag Gln 85
gaa Glu	ggt Gly	acc Thr 90	ttc Phe
atc Ile	gcg Ala	tgg Trp	
291			
ctg Leu	gtg Val 95	aaa Lys	ggt Gly
cgt Arg	gga Gly	tct Ser 100	cgt Arg
cac His	gaa Glu	ggt Gly 105	acc Thr
ttc Phe	acc Thr	agc Ser	
339			
gat Asp 110	gtg Val	agc Ser	agc Ser
tat Tyr	ctg Leu 115	gaa Glu	ggt Gly
cag Gln	gaa Glu 120	ggt Gly	acc Thr 125
387			
tgg Trp	ctg Leu	gtg Val	aaa Lys
ggt Gly 130	cgt Arg	gga Gly	tct Ser
cgt Arg	cac His 135	gaa Glu	ggt Gly
acc Thr 140	ttc Phe	acc Thr	
435			
agc Ser	gat Asp	gtg Val	agc Ser 145
agc Ser	tat Tyr	ctg Leu	gaa Glu
ggt Gly 150	cag Gln	gaa Glu 155	ttt Phe
atc Ile			
483			
gcg Ala	tgg Trp	ctg Leu 160	gtg Val
aaa Lys	ggt Gly	cgt Arg 165	tct Ser
cgt Arg	cac His	gaa Glu 170	ggt Gly
acc Thr	ttc Phe		
531			
acc Thr 175	agc Ser	gat Asp	gtg Val
agc Ser	tat Tyr 180	ctg Leu	gaa Glu
ggt Gly	cag Gln 185	gaa Glu 185	ttt Phe
579			
atc Ile 190	gcg Ala	tgg Trp	ctg Leu
gtg Val	aaa Lys 195	ggt Gly	cgt Arg
cac His	gaa Glu	ggt Gly 200	acc Thr 205
627			
ttc Phe	acc Thr	agc Ser	gat Asp
gtg Val 210	agc Ser	agc Ser	tat Tyr
ctg Leu	gaa Glu 215	ggt Gly	cag Gln
gaa Glu 220	ttc Phe		
675			
ttt Phe	atc Ile	gcg Ala	tgg Trp
ctg Leu 225	gtg Val	aaa Lys	ggt Gly
ggt Gly 230	cgt Arg	cac His	gaa Glu
ggt Gly 235	gaa Glu	ggt Gly	acc Thr
723			
acc Thr	ttc Phe	acc Thr 240	agc Ser
gat Asp	gtg Val	agc Ser	agc Ser 245
tat Tyr	ctg Leu	gaa Glu	ggt Gly
cag Gln 250	gaa Glu	ggt Gly	acc Thr
771			
gaa	ttt	atc	gcg
tgg	ctg	gtg	aaa
ggt	cgt	gga	tcc
taga			
811			

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Glu Phe Ile Ala Trp Leu Val Lys Gly Arg Gly Ser  
255 260 265

<210> 30  
<211> 3187  
<212> DNA  
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<220>  
<223> This sequence contains thirty-two copies of GLP-1(7-36)  
polypeptide

<220>  
<221> exon  
<222> (13)..(3183)

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1 5 10

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Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu Val  
15 20 25

aaa ggt cgt gga tct cgt cac gcg gaa ggt acc ttc acc agc gat gtg 147  
Lys Gly Arg Gly Ser Arg His Ala Glu Gly Thr Phe Thr Ser Asp Val  
30 35 40 45

agc agc tat ctg gaa ggt cag gcg gcg aaa gaa ttt atc gcg tgg ctg 195  
Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp Leu  
50 55 60

gtg aaa ggt cgt gga tct cgt cac gcg gaa ggt acc ttc acc agc gat 243  
Val Lys Gly Arg Gly Ser Arg His Ala Glu Gly Thr Phe Thr Ser Asp  
65 70 75

gtg agc agc tat ctg gaa ggt cag gcg gcg aaa gaa ttt atc gcg tgg 291  
Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala Trp  
80 85 90

ctg gtg aaa ggt cgt gga tct cgt cac gcg gaa ggt acc ttc acc agc 339  
Leu Val Lys Gly Arg Gly Ser Arg His Ala Glu Gly Thr Phe Thr Ser  
95 100 105

gat gtg agc agc tat ctg gaa ggt cag gcg gcg aaa gaa ttt atc gcg 387  
Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile Ala  
110 115 120 125

tgg ctg gtg aaa ggt cgt gga tct cgt cac gcg gaa ggt acc ttc acc 435  
Trp Leu Val Lys Gly Arg Gly Ser Arg His Ala Glu Gly Thr Phe Thr  
130 135 140

agc gat gtg agc agc tat ctg gaa ggt cag gcg gcg aaa gaa ttt atc 483  
Ser Asp Val Ser Ser Tyr Leu Glu Gly Gln Ala Ala Lys Glu Phe Ile  
145 150 155

gcg tgg ctg gtg aaa ggt cgt gga tct cgt cac gcg gaa ggt acc ttc 531  
Ala Trp Leu Val Lys Gly Arg Gly Ser Arg His Ala Glu Gly Thr Phe  
160 165 170

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atc Ile 190	gcg Ala	tgg Trp	ctg Leu	gtg Val	aaa Lys 195	ggt Gly	cgt Arg	gga Gly	tct Ser	cgt Arg 200	cac His	gcg Ala	gaa Glu	ggt Gly	acc Thr 205	627
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ttt Phe	atc Ile	gcg Ala	tgg Trp 225	ctg Leu	gtg Val	aaa Lys	ggt Gly	cgt Arg 230	gga Gly	tct Ser	cgt Arg	cac His	gcg Ala 235	gaa Glu	ggt Gly	723
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gaa Glu 255	ttt Phe	atc Ile	gcg Ala	tgg Trp	ctg Leu	gtg Val 260	aaa Lys	ggt Gly	cgt Arg	gga Gly	tct Ser	cgt Arg	cac His	gcg Ala	gaa Glu	819
ggt Gly 270	acc Thr	ttc Phe	acc Thr	agc Ser	gat Asp 275	gtg Val	agc Ser	agc Ser	tat Tyr	ctg Leu 280	gaa Glu	ggt Gly	cag Gln	gcg Ala	gcg Ala 285	867
aaa Lys	gaa Glu	ttt Phe	atc Ile	gcg Ala 290	tgg Trp	ctg Leu	gtg Val	aaa Lys	ggt Gly 295	cgt Arg	gga Gly	tct Ser	cgt Arg	cac His 300	gcg Ala	915
gaa Glu	ggt Gly	acc Thr	ttc Phe 305	acc Thr	agc Ser	gat Asp	gtg Val	agc Ser 310	agc Ser	tat Tyr	ctg Leu	gaa Glu	ggt Gly 315	cag Gln	gcg Ala	963
gcg Ala	aaa Lys	gaa Glu 320	ttt Phe	atc Ile	gcg Ala	tgg Trp	ctg Leu 325	gtg Val	aaa Lys	ggt Gly	cgt Arg	gga Gly 330	tct Ser	cgt Arg	cac His	1011
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gcg Ala 350	gcg Ala	aaa Lys	gaa Glu	ttt Phe	atc Ile 355	gcg Ala	tgg Trp	ctg Leu	gtg Val	aaa Lys 360	ggt Gly	cgt Arg	gga Gly	tct Ser	cgt Arg 365	1107
cac His	gcg Ala	gaa Glu	ggt Gly	acc Thr 370	ttc Phe	acc Thr	agc Ser	gat Asp	gtg Val 375	agc Ser	agc Ser	tat Tyr	ctg Leu	gaa Glu 380	ggt Gly	1155
cag Gln	gcg Ala	gcg Ala	aaa Lys 385	gaa Glu	ttt Phe	atc Ile	gcg Ala	tgg Trp 390	ctg Leu	gtg Val	aaa Lys	ggt Gly	cgt Arg 395	gga Gly	tct Ser	1203
cgt Arg	cac His	gcg Ala 400	gaa Glu	ggt Gly	acc Thr	ttc Phe	acc Thr 405	agc Ser	gat Asp	gtg Val	agc Ser	agc Ser	tat Tyr	ctg Leu	gaa Glu	1251
ggt Gly 415	cag Gln	gcg Ala	gcg Ala	aaa Lys	gaa Glu	ttt Phe 420	atc Ile	gcg Ala	tgg Trp	ctg Leu	gtg Val 425	aaa Lys	ggt Gly	cgt Arg	gga Gly	1299

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cgt Arg	gga Gly 495	tct Ser	cgt Arg	cac His	gcg Ala	gaa Glu 500	ggt Gly	acc Thr	ttc Phe	acc Thr	agc Ser 505	gat Asp	gtg Val	agc Ser	agc Ser	1539
tat Tyr 510	ctg Leu	gaa Glu	ggt Gly	cag Gln	gcg Ala 515	gcg Ala	aaa Lys	gaa Glu	ttt Phe	atc Ile 520	gcg Ala	tgg Trp	ctg Leu	gtg Val	aaa Lys 525	1587
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gat Asp	gtg Val	agc Ser 640	agc Ser	tat Tyr	ctg Leu	gaa Glu	ggt Gly 645	cag Gln	gcg Ala	gcg Ala	aaa Lys	gaa Glu 650	ttt Phe	atc Ile	gcg Ala	1971
tgg Trp 655	ctg Leu	gtg Val	aaa Lys	ggt Gly	cgt Arg	gga Gly 660	tct Ser	cgt Arg	cac His	gcg Ala	gaa Glu 665	ggt Gly	acc Thr	ttc Phe	acc Thr	2019
agc Ser	gat Asp	gtg Val	agc Ser	agc Ser	tat Tyr	ctg Leu	gaa Glu	ggt Gly	cag Gln	gcg Ala	gcg Ala	aaa Lys	gaa Glu	ttt Phe	atc Ile	2067

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acc Thr	ttc Phe	acc Thr	agc Ser	gat Asp 770	gtg Val	agc Ser	agc Ser	tat Tyr	ctg Leu 775	gaa Glu	ggg Gly	cag Gln	gcg Ala	gcg Ala 780	aaa Lys	2355
gaa Glu	ttt Phe	atc Ile	gcg Ala 785	tgg Trp	ctg Leu	gtg Val	aaa Lys	ggg Gly 790	cgt Arg	gga Gly	tct Ser	cgt Arg	cac His 795	gcg Ala	gaa Glu	2403
ggg Gly	acc Thr	ttc Phe 800	acc Thr	agc Ser	gat Asp	gtg Val	agc Ser 805	agc Ser	tat Tyr	ctg Leu	gaa Glu	ggg Gly 810	cag Gln	gcg Ala	gcg Ala	2451
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gaa Glu 830	ggg Gly	acc Thr	ttc Phe	acc Thr	agc Ser 835	gat Asp	gtg Val	agc Ser	agc Ser	tat Tyr 840	ctg Leu	gaa Glu	ggg Gly	cag Gln	gcg Ala 845	2547
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SEQ List.ST25

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